Respiratory Rate of Beef Steers

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Five ruminally fistulated steers received either a concentrate or roughage diet with or without p,p'-DDT (30 mg per kg body weight, daily) in a 4×4 Latin square experiment with 6-week experimental periods. Each period included 2 weeks adaptation to the experimental diets, after which ruminal ingesta was analyzed weekly and jugular blood was analyzed on weeks 4 and 6. DDT had little effect on ruminal pH, the proportions of individual volatile fatty acids, the concentration of total bacteria,

ecent studies have demonstrated that DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane] is converted to DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)ethane] by Proteus vulgaris (Barker et al., 1965) and Aerobacter aerogenes (Plimmer et al., 1968) under anaerobic conditions. Braunberg and Beck (1968) and Mendel and Walton (1966) demonstrated this conversion with microflora from the digestive tract of the rat. Miskus et al. (1965) showed that the same conversion took place when DDT was incubated anaerobically in fresh bovine ruminal fluid while ruminal fluid which had been boiled prior to incubation was inactive in the conversion of DDT to DDD. In other in vitro studies, Lemire and Fredette (1961) found that DDT stimulated the growth of Gram-negative rods, and Collins and Langlois (1968) demonstrated that Pseudomonas fluorensens was inhibited to varying degrees by 50-100 parts per million (ppm) of DDT in Trypticase Soy Broth. Harris et al. (1951) reported that nitrogen balance in lambs decreased from 3.1 to 0.1 g per day as the level of DDT in the diet increased from 0 to 42 ppm. A similar effect of DDT on the nitrogen balance of growing dairy calves was reported by Bohman et al. (1952).

To our knowledge, information is lacking concerning the extent to which DDT may change the microbial population in the rumen and which, in turn, may affect digestive fermentation and subsequent performance of the ruminant. The present study was designed to investigate the effect of feeding a moderately high level of p, p'-DDT on the ecology of the rumen, ruminal volatile fatty acids (VFA), electrocardiograph (EKG) patterns, and respiratory rate of steers receiving either a concentrate or roughage diet.

MATERIALS AND METHODS

Treatments. The study involved four treatments consisting of feeding either a 100% concentrate diet or an 81% roughage diet, each with or without the addition of p, p'-DDT (99+%, Aldrich Chemical Company, Inc., Milwaukee, Wis.) at a daily level of 30 mg per kg body weight. The concentrate diet, fed as a ground mixture, contained 80.0% cracked corn, 12.0% soybean oil meal, 5.0% molasses, 1.0% dicalcium

and the proportions of the different morphological types or Gram-stain of the bacteria. DDT appeared to reduce the total concentration of ruminal volatile fatty acids and protozoa, particularly when the concentrate diet was fed. The data support earlier findings that DDT is converted to DDD in the rumen. Electrocardiograph patterns and respiratory rates, obtained at the end of each experimental period, were not affected by DDT.

phosphate, 1.0% limestone, and 1.0% trace mineralized salt. In addition, a vitamin A and D supplement (20,000 USP units A per g and 2500 USP units of D per g) was added at the rate of 176 g per 1000 kg of feed. The roughage diet contained 65.7\% timothy hay, 15.2% alfalfa hay, 12.6% soybean oil meal, 5.0% molasses, 1.0% trace mineralized salt, and 0.5%dicalcium phosphate. The hay was ground through a 10-mm screen and the roughage diet was pelleted through a 10-mm die.

DDT was fed using soybean oil meal as a carrier. Fifteen kilograms of soybean oil meal were withheld from each 1000 kg of diet at the time of mixing, and aliquots of the withheld meal were placed on top of the diets at each feeding as either DDT-treated or untreated soybean oil meal. The treated meal was prepared by dissolving DDT in acetone, mixing the solution with the soybean oil meal, and evaporating the acetone.

Animals and Management. Five ruminally fistulated beef steers, averaging 350 kg body weight, were used as experimental animals. Four of the steers were assigned to the experimental treatments in an extra-period 4×4 Latin square design. The fifth steer was randomly assigned to a treatment series according to periods. This allowed one treatment during each period to contain two steers instead of one. The steers were housed in individual pens and fed twice daily an amount of feed equal to 0.75% of each steer's body weight. Water was available at all times.

To minimize the chances of DDT contamination across treatments, each of four pens was assigned an experimental treatment. The four steers initially assigned to the Latin square were then switched from pen to pen at the end of each experimental period of the Latin square. Since the fifth steer could not be switched, it remained in the same pen during the study and feed and water containers were cleaned at the end of each period. All pens were washed and bedded each morning and partially cleaned and bedded each evening.

Collection of Samples. The experimental periods were six weeks in length and samples of runnial ingesta were collected weekly, 4.5 to 5.5 hr after the morning feeding. Data from the 3-, 4-, 5-, and 6-week samples for each period were summarized and used in the statistical analysis. Samples of jugular blood were collected on weeks 2, 4, and 6 of each period, and data from weeks 4 and 6 were summarized. Prior to sampling at 6 weeks, EKG patterns were obtained as described by Rumsey *et al.* (1967a), except for the EKG

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	Experimental Diet				
Microbial Classifications ^a	100% Concentrate		81 % Roughage		
	Without DDT	Plus DDT	Without DDT	Plus DDT	Sī
Total bacteria per					
$ml \times 10^{-9}$	13.0	13.8	12.0	11.3	0.95
Gram –, %	73.0	80.4	73.6	73.9	2.26
Spirilla, %	1.8	3.1	2.5	2.0	0.48
Straight rod, %	20.7	27.3	21.5	15.5	3.69
Curved rod, %	13.9	15.5	14. 9	16.4	1.78
Cocci, %	36.6	34.5	34.7	40.0	4.33
Gram +, %	27.0	19.6	26.4	26.1	2.26
Straight rod, 7%	9.5	7.6	9.6	10.6	1.41
Straight rod, %°	5.3	2.2	2.5	4.5	1.27
Curved rod, %	2.1	1.8	2.2	1.8	0.92
Cocci, 7%	10.1	8.0	12.1	9.2	1.33
Total protozoa per					
ml \times 10 ^{-4 d}	28.1	14.0	14.2	13.2	3.40
Holotrich, % ^e	5.5	4.5	6.3	8.1	1.82
Dasytricha ruminantium, ^{o→}	3.3	2.9	3.9	6.0	1.34
Entodiniomorph, %	94.4	95.5	93.7	91.9	1.82
Diplodinium, %	26.3	27.2	24.8	24.9	2.54
Entodinium, %	27.2	28.2	27.5	25.3	2.04
Entodinium caudatum, %	4.1	5.6	4.0	5.2	1.49
Entodinium dentatum, %	3.8	2.6	5.9	2.3	1.29
Small entodiniomorph, % ⁷	33.2	32.0	32.4	33.9	3.30

Table I.Microbial Population of Ruminal Ingesta from Steers Fed a Concentrate or Roughage DietWith and Without p, p'-DDT

^a All means are an average of 24 samples, except the roughage plus DDT means, which are an average of 28 samples. ^b Rods greater than 1 μ in length. ^c Rods 1 μ or less in length. ^d The concentration of total protozoa was affected by type of diet (P < 0.05). ^e Includes Isotricha prostoma, Isotricha intestinalis, and Dasytricha ruminantium. ^f Small entodiniomorph were similar in size to the smaller forms of Entodinium and Diplodinium. However, it was not possible to positively identify the genera to which these organisms belonged, so they were grouped separately.

leads. In this study, patterns of only one bipolar lead were obtained. Two silver-coated plates $(5.1 \times 7.6 \text{ cm} \text{ each})$ were placed on the lower part of the chest immediately caudal to the olecranon, one plate on each side of the animal. The plates were held in place by an elastic band which was stretched around the animal's chest, and electrode paste was placed between the skin and plates. At the same time, respiratory rate was determined by using a bellows-type pneumograph.

Following each 6-week sample collection, the rumen of each steer was manually emptied *via* the rumen fistula for the determination of the volume, weight, and dry matter content of ruminal ingesta. In an attempt to shorten ruminal adaptation to the experimental diets, the ruminal contents from each of the four initial steers were then placed in the rumens of the steers scheduled to receive that treatment next. For example, the ruminal ingesta of the steer receiving the concentrate diet plus DDT during the first period was switched to the steer scheduled to receive that same treatment during the second period. It was not possible to switch the ruminal contents of the fifth steer. In addition, 3–5 g of perianal fat were surgically removed at the end of each 6-week period for DDT residue analysis.

Microbiology. One gram of unstrained well-mixed ruminal ingesta was added to 1 ml of formalin and 2 ml of saline. Total bacterial numbers were determined as described by Slyter and Putnam (1967) and protozoal counts were obtained by the procedure of Slyter *et al.* (1964). Smears of the ruminal ingesta samples were diluted 1 to 10 in the dilution fluid of Bryant and Robinson (1961) and Gram-stained. The Gram reaction and morphology of the bacteria were determined using a phase microscope and oil immersion. One hundred bacteria were counted for each sample assayed.

Analytical. A portion of each ruminal ingesta sample was strained through cheesecloth and pH was determined on the fluid. One hundred milliliters of strained fluid were

mixed with 20 ml of 25% metaphosphoric acid, cooled, centrifuged, and VFA were determined on the supernatant according to the gas-liquid chromatography procedure of Baumgardt (1964).

Forty milliliters of the prepared ruminal fluid, 40 ml of heparinized blood, and the perianal fat samples were each analyzed for DDT, DDD, and DDE [1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethylene] using the procedures for the extraction and clean-up of samples as outlined by Mills (1961) and gas-liquid chromatography (Rumsey *et al.*, 1969). Distinction was not made between the *ortho-para* and *parapara* isomers; however, data in the literature would indicate that the *para-para* isomer, which was fed in this study, is converted to little, if any, *ortho-para* isomer in the animal (Klein *et al.*, 1964).

RESULTS

Rumen Ecology. The data obtained from the fifth steer were similar to the initial four steers for all parameters in this study and were therefore included in the statistical analysis (Steel and Torrie, 1960). The influence of diet and DDT on the concentrations of ruminal protozoa and bacteria and their general classifications is shown in Table I. The average concentration of protozoa in ruminal ingesta was greater (P < 0.05) when the concentrate diet was fed as opposed to the roughage diet, and the concentration was less when DDT was added to the diet (P < 0.065), particularly when DDT was fed with the concentrate diet. The diet X DDT interaction for total protozoa was significant at P <0.10. The individual protozoal species and genera were not significantly changed due to treatment except for the proportion of *E. dentatum* (P < 0.09) which increased with time when DDT was not fed (3.2 to 7.1 % for weeks 3 and 6, respectively) and decreased with time when DDT was fed (3.6 to 1.9% for weeks 3 and 6, respectively). Among the bacteria of a

Table II. Composition of Ruminal Ingesta of Steers Fed a Concentrate or a Roughage DietWith and Without p, p'-DDT

		Experimental Diet				
	100% Co	100% Concentrate		81 % Roughage		
Ruminal Ingesta Measurements ^a	Without DDT	Plus DDT	Without DDT	Plus DDT	Sīx	
Volume, 1 ^{b.e.d}	31.2e	31.2°	47.2 ¹	40.00	1.53	
Weight, kg ^{b,c,d}	30.9°	30.Ge	46.4/	40.9/	1.34	
Dry matter, $\%^b$	6.1	5.7	11.1	8.7	0.78	
$p\mathbf{H}^{h}$	6.2	6.3	6.4	6.5	0.07	
Total VFA, mm/1.	73.9	63.8	63.9	61.2	2.99	
Acetic, M $\%^b$	53.3	54.2	65.1	64.7	1.05	
Propionic, M $\frac{0.7b}{2}$	23.9	23.7	20.2	20.1	0.27	
Isobutyric, M %	2.9	3.1	2.5	2.6	0.15	
Butyric, M % ^b	13.7	12.7	10.4	10.8	0.70	
Isovaleric, M $^{\circ 7h}_{\circ 0}$	4.9	4.8	1.2	1.5	0.20	
Valeric, M $\%^{b}$	1.4	1.3	0.8	0.8	0.11	

^a The means represent an average of 6 measurements for ruminal fill and 24 for pH and VFA, except the roughage plus DDT means, which are an average of 7 and 28, respectively. ^b Ruminal measurements were affected (P < 0.01) by diet. ^c Ingesta volume and weight were affected (P < 0.05) by the addition of DDT to the diet. ^d Significant diet \times DDT interaction (P < 0.05) was obtained for ingesta volume and weight. ^{e.f.a} Means with different superscripts within each row are different (P < 0.05) as determined by Tukey's w procedure (Steel and Torrie, 1960). ^h Ruminal pH was affected (P < 0.05) by diet.

particular Gram-reaction and morphology, only the short $(<1.0 \ \mu)$ Gram-positive rods were significantly (P < 0.07) affected by DDT. The percent of these bacteria among the total bacterial population decreased when DDT was fed with the concentrate diet and increased when DDT was fed with the roughage diet. The remaining morphological groups of bacteria of a given Gram-reaction, the proportion of Gramnegative and positive bacteria, or the total number of bacteria per milliliter of ruminal ingesta were not affected by treatment.

Ruminal Ingesta, pH, and VFA. The differences in rumen fill and the pH and VFA content of ruminal fluid are shown in Table II. The volume and weight of ruminal ingesta were greater (P < 0.01) when the roughage diet was fed and less (P < 0.05) for roughage plus DDT than for roughage with no DDT. However, the difference between roughage with and without DDT for ingesta weight was not significant (P < 0.05) when Tukey's w procedure (Steel and Torrie, 1960) was used to compare treatment means. Similar trends were obtained for ingesta dry matter, but the differences were statistically significant only for diet (P < 0.01).

Ruminal pH (P < 0.05) and the molar proportion of acetic acid (P < 0.01) were less while the molar proportions of pro-

Table III. Respiratory Rate, Heart Rate and EKG Patterns of Steers Fed a Concentrate or Roughage Diet With and Without p, p'-DDT

	100 % Concentrate		81 % Roughage			
Physiological Measurements ^a	With- out DDT	Plus DDT	With- out DDT	Plus DDT	Sĩ	
Respiratory rate,						
respirations per min	43	39	40	41	5.0	
Heart rate, beats						
per min ^b	78	73	64	67	3.9	
EKG intervals						
P, sec	0.08	0.08	0.08	0.08	0.004	
P-R, sec	0.16	0.17	0.18	0.18	0.006	
QRS, sec	0.08	0.10	0.10	0.09	0.005	
Q-T, sec	0.32	0.33	0.33	0.33	0.009	
T, sec	0.12	0.13	0.13	0.13	0.008	

^a All means are an average of 6 determinations except for the roughage plus DDT means, which are an average of 7. ^b Heart rate was affected (P < 0.05) by diet.

pionic, isobutyric, butyric, isovaleric, and valeric acids were greater (P < 0.01) when the concentrate diet was fed. Feeding DDT did not affect ruminal pH or the relative amounts of the individual VFA. The total concentration of VFA was greater (P < 0.075) when the concentrate diet was fed and was reduced (P < 0.065) when DDT was fed with the diets. The reduction was more pronounced with the concentrate diet than with the roughage diet, but the diet X DDT interaction was not statistically significant (P < 0.10).

Physiological Measurements. Table III contains data showing the influence of diet and DDT on respiratory rate, heart rate, and the various intervals of the EKG patterns. Under the conditions of this study, these physiological measurements were not affected by treatment except for heart rate, which was increased (P < 0.05) when the concentrate diet was fed.

DDT Residues. The results of the residue analyses of ruminal fluid, jugular blood, and perianal fat tissue are shown in Table IV. Feeding DDT increased (P < 0.05) the total residue content in the ruminal fluid samples. Although the total amount of DDT consumed was similar for both the concentrate and roughage diets, the average level of total residues measured in ruminal fluid was higher when the concentrate diet was fed (P < 0.07). This difference was greater than what could be accounted for by the differences in ruminal ingesta volumes.

The total DDT residue content of ruminal fluid samples and the relative amounts of DDT and DDD were similar when the steers consumed either diet without DDT. When DDT was fed, the relative amount of DDD in the ruminal fluid was greater, although not significantly different, than the amount of DDD in ruminal fluid when DDT was not fed. Measurable amounts of DDE (less than 1 ppb) were not found in the samples of ruminal fluid.

The total DDT residue content of the blood was somewhat greater when DDT was fed. Although not statistically significant (P < 0.10), the increase in the total DDT residue content of blood appeared to be greater when DDT was fed with the roughage diet than when fed with the concentrate diet. A similar trend was noted for the relative proportion of DDT. The relative percent of DDD in the blood increased (P < 0.05) when DDT was added to the concentrate diet, but was not changed when DDT was added to the roughage diet. The relative proportion of DDE in the blood was less (P <

	Experimental Diet					
	100% Co	100% Concentrate		oughage		
DDT Residues	Without DDT	Plus DDT	Without DDT	Plus DDT	Sīx	
			Ruminal Ingesta ^a			
Total residues, ppm ^b DDT, relative % DDD, relative %	0.67° 48.2 51.8	5.58 ^{<i>d</i>} 39.3 60.7	0.69° 45.7 54.3 Blood®	1.44 ^{c,d} 36.0 64.0	0.96 5.47 5.47	
Total residues, ppb DDT, relative % DDD, relative % DDE, relative %	53.9 21.2 23.7° 55.1°	61.8 23.5 44.7 ^d 31.8 ^d	64.4 21.9 29.0 ^{c.d} 49.1 ^{c.d} Perianal Fat ^g	69.2 28.1 28.2 ^{c.d} 43.7 ^{c.d}	5.14 4.51 3.73 3.61	
Total residues in whole tissue, ppm ^b Total residues in extracted fat, ppm ^b DDT, relative % DDD, relative %	52.1 87.7 37.3 ^c 52.4	95.4 135.2 47.9 ^{e.h} 47.3	53.9 95.8 40.3 ^c 52.5	100.3 170.8 58.5 ^{d,h} 34.9	16.21 23.24 3.35 4.72	
DDE, relative $\%^b$	10.3	4.8	7.2	6.6	1.19	

Table IV.	DDT, DDD, and DDE in Ruminal Fluid, Blood, and Perianal Fat of Beef Steers Fed a
	Concentrate or Roughage Diet With and Without p, p' -DDT

^a Each mean is an average of 24 samples, except for the roughage plus DDT means which are an average of 28 samples. ^b Blood DDE (P < 0.01) and blood DDD, ruminal ingesta total residues, perianal fat total residues, and perianal fat DDE (P < 0.05) were affected by the addition of DDT to the diet. ^{c,d,h} Means with different superscripts within each row are different (P < 0.05), as determined by Tukey's w procedure. ^e Each mean is an average of 12 samples, except for the roughage plus DDT means, which are an average of 14 samples. ^f Addition of DDT to the concentrate diet affected the relative amounts of blood DDD and DDE more (P < 0.05) than with the roughage diet. ^g Each mean is an average of 6 samples, except for the roughage plus DDT means, which are an average of seven samples.

0.01) when DDT was fed and this difference was greater (P < 0.05) when the concentrate diet was fed with and without DDT.

Total residues in the perianal fat were significantly greater (P < 0.05) at the end of each 6-week period when DDT was fed than when DDT was not fed. Although the diet X DDT interaction was not statistically significant, the magnitude of residue accumulation appeared greater when the roughage diet was fed. A similar change in the relative proportion of DDT occurred and the increase in the relative percent of DDT when the roughage diet plus DDT was fed was significant (P < 0.05). The increase in the proportion of DDT was accompanied by decreases in the proportion of DDD and DDE. The decrease was statistically significant for DDE (P < 0.05) but not for DDD.

DISCUSSION

The literature contains many studies related to the biological fate of DDT in animals. It has been shown that the ortho-para isomers are changed to the para-para isomers (Klein et al., 1964), that DDD and DDE are formed after the administration of DDT, and that these compounds accumulate in adipose tissue (Fries and Kane, 1967; McCully et al., 1966; Rumsey et al., 1969; Rumsey et al., 1967b; Witt et al., 1966). Experimental evidence indicates that DDD and DDE are formed from DDT by separate mechanisms (Peterson and Robinson, 1964) and DDD is further metabolized to DDA [bis(p-chlorophenyl)ethanoic acid] and other water soluble metabolites which are excreted in the urine (Pinto, 1963). However, knowledge of the location, mechanism, and extent of DDT metabolism is incomplete. It has been shown that the liver is capable of converting DDT to DDD and that DDE apparently arises from some other site in the body (Whiting et al., 1968). The conversion of DDT to DDD has also been demonstrated in isolated cultures of microorganisms, in the gastrointestinal tract of rats, and in ruminal ingesta (Barker et al., 1965; Braunberg and Beck, 1968; Cope, 1965; Mendel and Walton, 1966; Plimmer *et al.*, 1968; Wedemeyer, 1966).

The present data support the thesis that DDT is not inert in the rumen but is converted to DDD by the ruminal microorganisms. Concurrently, it appears that DDT, fed in this study at a level much higher than would normally be encountered, had little effect on ruminal bacteria population as determined by morphology and the Gram reaction, total bacterial counts, proportions of protozoal species, and the proportions of VFA. Although little work has been reported relative to the effect of DDT on microorganisms, Braunberg and Beck (1968) reported that they did not find any differences in the numbers of Gram-negative rods in the gastrointestinal tract of rats fed DDT compared to those fed a control diet. Earlier, Lemire and Fredette (1961) had suggested that DDT stimulated the growth of Gram-negative rods. In the present study, the proportion of small Gram-positive rods was affected by DDT, but the observed changes were not consistent. It is not clear why these small Gram-positive organisms decreased when the concentrate diet was fed and increased when the roughage diet was fed, but it would appear to be more than a direct effect of DDT, since the changes depended on the type of diet that was fed.

DDT decreased protozoal numbers and the total concentration of VFA, particularly when the cattle were fed the concentrate diet. This agrees with data that has shown chemical agents to cause greater toxicity to concentrate-fed ruminal microbial populations than to populations fed roughage (Slyter and Wolin, 1967). Lowered protozoal numbers may have also been a causative factor in the reduction of nitrogen balance observed by Harris *et al.* (1951) with DDT fed lambs. Abou Akkada and El-Shazly (1964) reported lower nitrogen retention in lambs free of ciliate protozoa.

Since DDE, DDD, and DDT have an affinity toward similar biological media, the presence of DDE in the blood but not in the rumen may suggest that little if any DDT and DDD were reabsorbed or recycled into the rumen from the internal

body. In contrast, other chlorinated hydrocarbons have been shown to be recycled (Wilson et al., 1968). This is an important consideration when studying the absorption and excretion of pesticide residues and warrants further study on a quantitative basis. If there is no reabsorption or recycling, then our data would indicate that DDT is converted to DDD in the rumen at a greater rate than the disappearance of DDD from the rumen. This is based on the findings that the residual proportions of DDT in ruminal ingesta decreased and DDD tended to increase when the steers received DDT. Apparently, even at this relatively high daily residue intake of 30 mg per kg body weight, the ability of ruminal ingesta to convert DDT to its less toxic metabolite DDD was not overloaded. An investigation of the further metabolism of DDD was not made in this study, but other workers have reported evidence that DDD may undergo further metabolism by Proteus vulgaris, a microorganism isolated from the gastrointestinal tract of the mouse (Barker and Morrison, 1965).

Although this study was not designed to study tissue residues, we felt certain changes in tissue residues were of interest. Based on our data, feeding the roughage diet was associated with a higher level of total DDT residues in depot fat. This may have been due to a decrease in the total fat pool of the body when the roughage diet was fed; however, a decreased fat pool was not apparent on the basis of weight change over each 6-week experimental period. Weight changes were similar on both diets. Diet may have affected the rate of absorption of residues from the rumen, based on the fact that total residue concentration in the ruminal ingesta was less when DDT was fed with roughage compared to concentrate and, at the same time, somewhat greater in the blood

The feeding of p, p'-DDT with the roughage diet compared to the concentrate diet also appeared to be associated with a larger proportion of DDT in the fat tissue relative to DDD and DDE. It was also noted that a slightly smaller proportion of the total residue content of ruminal ingesta was present as DDT when the roughage diet was fed, but a trend was noted in the blood similar to that noted in fat tissue. This would suggest that the type of diet influenced the absorption and/or conversion of DDT at the rumen level, and that this dietary effect influenced depot fat residues. The differences in the proportion of DDE in the blood suggests that the type of diet may elicit an effect on the metabolism of DDT elsewhere in the body in addition to what occurs at the rumen level. More research is needed to define the various

fractions of ingesta with which residues are associated, the rate of passage through the digestive tract, and the site of residue absorption from the tract in order to obtain a clearer understanding of the fate of ingested chlorinated hydrocarbons such as DDT.

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